July 27, 1949.

Dear Cavalli:

First, let me thank you for your kindness in sending the Hfr strains, which arrived about a week ago in excellent condition. For convenience, I will refer to your "58-161 Ny^r [Hfr]" as W-1033.

As you may imagine, I have hastened to try to confirm your findings, and to a considerable extent have done so. My earlier "kinetical" experiments were conducted in deep minimal agar. I now compared 58-161 with W-1033 at various cell densities spread with a constant excess (ca. 3 x 10^8) of W-677 on minimal agar + B_1. I was surprised to find that under these conditions, even 58-161 gives extraordinarily high yields of prototrophs:

	-log dilution	58-161	W-1033 [X W-677]
1 ml ~ 10^9	0	1000+	....
	3	11	1000+
	4	2	200
	5	4	20+
	6	-	4

58-161 does not seem to give a linear cutoff, but one can adjudge that W-1033 is roughly 100x as efficacious. These quantitative experiments are, of course, largely vitiated by the formation of microcolonies whose size probably varies inversely with the density of inoculation. However, this may depend a great deal on the quality of agar used, and on the chemical purity of the reagents. Howbeit, I still have not approached an efficacy of prototroph formation of the order of 10%-100% as you achieved. From these experiments, also, I could not conclude that the difference between 58-161 and W-1033 related specifically to sexual potency. W-1033 might merely give larger microcolonies as a result of syntrophic efficacy, or might be more motile. However, stimulated by your findings, I have tried another approach which has given astonishing results. Earlier, I had tried to see whether complementary segregants could not be identified by conducting "crosses" not a selective minimal, but on an indicator complete medium. For example, I grew Lac_1- and Lac_2-, (each of which is lactose negative, but which recombine readily to give Lac+,) together in a complet medium, then plated out large numbers of cells on EMB lactose to look for any sectors of Lac+. I did not find any with previous stocks, but decided to see whet recombination with Hfr was so frequent that growth-selection might be dispensed with. The material first available was W-1033 itself, which is Lac+, so a slightl different approach was tried. W-1033 was inoculated heavily with W-677 into Pennassay broth, and then after 24-48 h. the mixture was diluted and plated on various EMB agars. The colonies were then tested on other sugars to look for possible recombinations of fermentative character. One such experiment is tabulat [W-1033 + W-677] plated on Xyl EMB. 44 Xyl+ colonies tested on Mal EMB. 39 were Mal+ (parental); 5 were Mal- (recomb.) Similarly, of 35 Xyl-, 34 Mal- (par.) and 1 Mal+ (rec.) The six apparent recombinants were then thoroughly diagnosed:

Exp. 583C

Culture	Lac	Mal	Xyl	Gal	Mal	V_1	Nutr.	
W-677	+	-	-	-	-	r	TLB_1	
W-1033	+	+	+	+	+	s	M [B]	B not scored throughout
1	+	-	+	-	-	r	L	
2	+	-	+	-	+	s	MT	
3	-	-	+	-	+	r	TLB_1	
4	-	-	+	-	-	s	B_1	
5	-	-	+	+	+	r	MTL	
6	-	+	-	-	-	s	TLB_1.	

Thus there were 6 recombinants with respect to Xyl and Mal out of 79 tests! And what a wealth of material!

If any of these selections are Hfr (and I have preliminary indications that some are) they will provide excellent material for another technique of selection, namely the use of EMB-two sugars. For example, if one parent is Lac+Mal- and the other Lac-Mal+ then both parents will give a pure + reaction on EMB-[Lac+Mal]. However, recombinants may be Lac-Mal- and give a - reaction. I am especially interested to use this technique to find sectored colonies which may represent the segregation of single zygote cells. To this end, I have also irradiated W-1033 (and it appears as expected to be somewhat resistant to uv) and have picked up six new lactose-negative mutants. One is Lac_3-, and Ismay hope that one or more of the others is also distinct from Lac_1-, which would allow plating on EMB Lac to look for Lac+ recombinants as mentioned above. I have yet to re-study 58-161 from the viewpoint of Exp. 583C above, but myspast experience does not readily admit of the possibility that it will behave in a similar manner. To my mind, this kind of evidence would clinch the comclusion that in Hfr you truly have isolated a more sexually active strain. The high frequency of recombinants also strongly heightens the possibility that the sexual fusion may be cytologically demonstrable, for which project you certainly carry my best wishes. I have also just crossed 1033 into Hfr stocks and isolated some Lacy diploids. From this material also, it should be possible to select good Hfr strains to pursue our investigations. I will send you whatever material comes to hand that might be useful.

Just now, your letter has been received. Thank you for enclosing the MSS. May I request, first of all, that any reference to the discovery of recombination include Professor E. L. Tatum, whose role in the initiation of the work is not adequately indicated even by his joint authorship on some of the papers.

Your letter gave some detailed data on W-1033 x W-677 which is not in good agreement with mine. E.G., 2.5×10^3 W583 + 2×10^3 W1033 gave 510 prototrophs in your experiment, but only a (calculated) 8 in mine. The thiamin agar plates I used were not more than a day old, and quite moist. I don't believe that the difference can be a matter of strain, but it is conceivable. I do not notice in your MS any reference to a comparison of 58-161 with Hfr like that on the first page of thei letter. I would be extremely interested to learn whether you have found as high an efficiency as I did with 58-161. Finally, it may be suggested that all present 58-161 cultures are heterogeneous for Hfr. It might be worthwhile to establish a number of single colony subcultures and examine their variance in prototroph production. I've done this once without interesting result (Cf. p. 519 Genetics 1947), but it might well be repeated. It was extremely gratifying to note the close coincidence in many details between our data on various segregations. One always has an uneasy feeling about the reproducibility of results in another's hands.

I do not think that the discrepancies in my observations on Hfr are especially important, but they might be worth some further inspection. Your comment on the effect of leucine (fig. 1) is readily explained: most samples are fairly heavily contaminated, especially with methionine. This is usually quite apparent on the plate because the background growth is much heavier. I have never used frequency of prototrophs as an index of crossing-over, except qualitatively with B_1.

No less interesting is the discovery of a coli strain which can cross with K-12. I think that itwould be especially interesting to ascertain the nature of the genic differences between such strains, and in particular the genic determination of the serological distinctions between them. Kauffmann, in Copenhagen, has rather thoroughly worked out the serology of the coli group, and I strongly recommend that you send him the parental and a number of recombinant cultures for serological comparison. I have been looking for some time for other crossable strains, both in coli and in Salmonella, and even resorted to the use of "natural" nutritional deficiencies in the latter. However, I have come to the conclusion that with the selective methods now available, especially the penicillin method, it is more economical in the long run to start with well defined, easily cultivated, strains and to induce mutants in them. If a short cut is needed, I think that the use of drug-resistance (using several agents) as a selector is available. It has worked with K-12. In Salmonella, using biochemical mutants, some meagre evidence of recombination in typhimurium has been picked up, but the same peculiarity, the persistence en bloc of parental characters, has plagued us. Also, most Salmonella are lysogenic, and mutual lysis, or suicide, has complicated the results condiderable. It should be mentioned that a third or more of coli strains also carry latent phages (K-12 does,for example) and that this must be taken into account in interpreting "recombinations". However, unless coli 123 is unstable Lac+, the recovery of Lac- prototrophs may be taken as nearly conclusive.

Concerning the oppositional character of the Hfr effect, I am not clear whether the cross of the derived Ar- x B_1-, both of which are presumably Hfr, gave the same high rate of recombination. I.E., how much is "rather high" (p. 10).

As to starting from scratch, we have already done this, and developed several new systems of mutants from K-12. To map them all completely, however, seems rather hopeless, however. The main question that I hope to answer is whether the segregatic peculiarities of K-12 stocks depend on structural heterozygosity, or some intrinsic pattern of meiotic instability. That alterations in pattern do occur is quite clear from the unique segregation patterns that have lately shown up. For example, I have a TLB_1- Lac- Mal- segregant which when crossed with 58-161 gives over 95% Lac+ and Mal+, instead of the expected excess of - recombinants.

You will hear from me again on the controls of the comparison of W1033.

May you enjoy your vacation,

Sincerely,

Joshua Lederberg.